10/518432

New Application Preliminary Amendment Attorney Dockt: P70329US0

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## Amendments to the Claims:

The listing of claims will replace all prior versions, and listings of claims in the application:

## **Listing of Claims:**

- 1. (original) A recombinant, purified, or isolated polypeptide comprising an amino acid sequence selected from
- (a) the sequence of SEQ ID No: 1;
- (b) a functionally equivalent variant of the sequence of SEQ ID NO: 1 which has greater than 77% amino acid sequence identity with SEQ ID NO: 1; and
- (c) a functionally equivalent fragment of a polypeptide defined in (a) or (b).
- 2. (original) A recombinant, purified, or isolated polypeptide comprising an amino acid sequence selected from
- (a) amino acids 20 to 235 of SEQ ID NO: 1
- (b) a functionally equivalent variant which has greater than 77% amino acid sequence identity with amino acids 20 to 235 of SEQ ID NO: 1; and
- (c) a functionally equivalent fragment of a polypeptide defined in (a) or (b).
- 3. (original) A polypeptide as claimed in claim 2 wherein the sequence has greater than 90% identity with SEQ ID NO: 1.
- 4. (original) A polypeptide as claimed in claim 2 wherein the sequence has greater than 99% identity with the sequence of amino acids 20 to 235 of SEQ ID NO: 1.
- 5. (original) A polypeptide as claimed in claim 2 wherein the sequence is that of amino acids 20 to 235 of SEQ ID NO: 1.

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- 6. (currently amended) A polypeptide as claimed in <u>claim 1 any one of claims 1 to</u> 5 which is obtainable from a bacterium.
- 7. (currently amended) A polypeptide as claimed in <u>claim 1 any one of claims 1 to</u> 5 which is obtainable from *Mycobacterium avium* subspecies *paratuberculosis*.
- 8. (currently amended) A polypeptide as claimed in <u>claim 1</u> any one of claims 1 to 5 which is obtainable from a heterologous host transformed with a polynucleotide which encodes said polypeptide or functionally equivalent variant or fragment thereof wherein said host is capable of expressing said polypeptide.
- 9. (original) A polypeptide as claimed in claim 8 wherein the host is *E coli*.
- 10. (currently amended) A genetic construct comprising
- (a) a promoter sequence;
- (b) an open reading frame polynucleotide encoding a polypeptide as claimed in <u>claim 1</u> any one of claims 1 to 5;
- (c) a termination sequence.
- 11. (original) A recombinant, purified, or isolated polynucleotide comprising the sequence of SEQ ID NO: 2 or a variant thereof encoding either the polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a functionally equivalent fragment of said polynucleotide.
- 12. (original) A recombinant, purified or isolated polynucleotide with a nucleotide sequence complementary to the polynucleotide of claim 11.
- 13. (currently amended) One or more oligonucleotide or polynucleotide primers capable of amplifying a polynucleotide which encodes a polypeptide as claimed in claim 1 or claim 2 in a Polymerase Chain Reaction or other polynucleotide amplification method.

- 14. (currently amended) A purified or isolated antibody capable of binding a polypeptide as defined in claim 4 or 5.
- 15. (currently amended) A vaccine composition comprising a polypeptide as claimed in <u>claim 1</u> any one of claims 1 to 8 and an acceptable diluent, carrier, excipient, or adjuvant, said polypeptide being present in an amount sufficient to generate a protective immune response to *Mycobacterium avium* subspecies *paratuberculosis* infection.
- 16. (currently amended) A diagnostic composition for use in detecting the presence of *Mycobacterium avium* subspecies *paratuberculosis*, wherein said composition comprises a polypeptide as claimed in <u>claim 1 elaims 1 to 8</u>.
- 17. (currently amended) A diagnostic composition for detecting the presence of *Mycobacterium avium* subspecies *paratuberculosis*, wherein said composition comprises a polynucleotide according to claim 11 or claim 12.
- 18. (currently amended) A diagnostic composition for detecting the presence of *Mycobacterium avium* subspecies *paratuberculosis* comprising at least one oligonucleotide or polynucleotide primer capable of amplifying a polynucleotide which encodes a polypeptide as claimed in <u>claim 1 any one of claims 1 to 8</u> in a Polymerase Chain Reaction or other polynucleotide amplification method.
- 19. (original) A diagnostic composition for detecting the presence of *Mycobacterium* avium subspecies paratuberculosis comprising an antibody according to claim 14.
- 20. (currently amended) A method of detecting Johne's disease including preclinical Johne's disease in an animal comprising contacting either the animal or a sample from the animal with a polypeptide as claimed in <u>claim 1 any one of claims 1 to 8</u> and detecting an immune response indicative of the presence of *Mycobacterium avium* subspecies *paratuberculosis*.
- 21. (original) A method according to claim 20 wherein the response is a delayed-type hypersensitivity response.

- 22. (currently amended) A method according to claim 20 wherein said detecting comprises detecting the presence of antibodies that bind a recombinant, purified, or isolated polypeptide comprising an amino acid sequence selected from (a) amino acids 20 to 235 of SEQ ID NO: 1; (b) a functionally equivalent variant which has greater than 99% amino acid sequence identity with amino acids 20 to 235 of SEQ ID NO: 1; and (c) a functionally equivalent fragment of a polypeptide defined in (a) or (b)as claimed in claim 4 or 5.
- 23. (original) A method according to claim 22 wherein the detection of the presence of antibodies is by ELISA, radioimmunoassay or Western blotting.
- 24. (currently amended) A method of detecting Johne's disease including preclinical Johne's disease in an animal comprising contacting a sample from the animal either with a purified or isolated antibody capable of binding a recombinant, purified, or isolated polypeptide comprising an amino acid sequence selected from (a) amino acids 20 to 235 of SEQ ID NO: 1, (b) a functionally equivalent variant which has greater than 99% amino acid sequence identity with amino acids 20 to 235 of SEQ ID NO: 1, and (c) a functionally equivalent fragment of a polypeptide defined in (a) or (b); according to claim 14 or a composition comprising an antibody specific to the recombinant, purified, or isolated polypeptide comprising an amino acid sequence selected from (a) amino acids 20 to 235 of SEQ ID NO: 1, (b) a functionally equivalent variant which has greater than 99% amino acid sequence identity with amino acids 20 to 235 of SEQ ID NO: 1, and (c) a functionally equivalent fragment of a polypeptide defined in (a) or (b); defined in claim 4 or claim 5 and detecting a polypeptide which binds to the antibody.
- 25. (original) A method according to claim 24 wherein the presence of bound antibody is determined by ELISA, radioimmunoassay or Western blotting.
- 26. (original) A method according to claim 24 for detecting the presence of *Mycobacterium avium* subspecies *paratuberculosis* at a preclinical phase of Johne's disease.

- 27. (currently amended) A method of detecting Johne's disease including preclinical Johne's disease in an animal comprising contacting a sample from the animal with a composition comprising of at least one oligonucleotide or polynucleotide primers capable of amplifying a polynucleotide which encodes a polypeptide as claimed in claim 4 or elaim 5 in a polynucleotide amplification method and detecting the amplification product.
- 28. (original) A method as claimed in claim 27 wherein the polynucleotide amplification method is a polymerase chain reaction method.
- 29. (original) A method according to claim 22 for detecting the presence of *Mycobacterium avium* subspecies *paratuberculosis* at a preclinical phase of Johne's disease.
- 30. (currently amended) A method of detecting Johne's disease in an animal comprising contacting a sample from the animal with a composition comprising a polynucleotide capable of binding to a polynucleotide which encodes a polypeptide as claimed in claim 4 or claim 5.
- 31. (original) A method according to claim 30 wherein said polynucleotide is detectably labeled.
- 32. (original) A method according to claim 31 wherein said detectable label is a radioisotope or fluorescent tag.
- 33. (currently amended) A method of prophylactically or therapeutically treating an animal against Johne's disease which comprises administering to an animal a polypeptide as claimed in <u>claim 1 any one of claims 1 to 5</u> to produce a protective immunological response in the animal.
- 34. (original) A method according to claim 33 which is a therapeutic method.
- 35. (original) A method according to claim 33 which is a prophylactic method.

- 36. (original) A method of vaccinating against Johne's disease which comprises administering to an animal a vaccine composition as claimed in claim 15 in an amount sufficient to produce a protective response.
- 37. (original) A method according to claim 36 wherein said administration is performed on a single occasion.
- 38. (original) A method according to claim 36 wherein said administration is performed on more than one occasion.
- 39. (original) A method as claimed in claim 3 wherein 0. 1-100011G/KG is administered of a recombinant, purified, or isolated polypeptide comprising an amino acid sequence selected from
- (a) the sequence of SEQ ID No: 1;
- (b) a functionally equivalent variant of the sequence of SEQ ID NO: 1 which has greater than 77% amino acid sequence identity with SEQ ID NO: 1;
- and (c) a functionally equivalent fragment of a polypeptide defined in (a) or (b).
- 40. (original) A method as claimed in claim 39 wherein 5-500μG/KG of the polypeptide is administered.
- 41. (currently amended) A kit for use in detecting the presence of *Mycobacterium* avium subspecies paratuberculosis comprising at least two of the following:
- a polypeptide as claimed claim 1 in any one of claims 1 to 8;

an antibody that binds said polypeptide, and

- a reagent for determining antigen-antibody binding.
- 42. (currently amended) A host cell transformed with a polynucleotide of claim 11 or claim 12.

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- 43. (original) A vector comprising the construct as claimed in claim 10.
- 44. (original) A host cell incorporating a construct of claim 10.
- 45. (original) A host cell incorporating a vector as claimed in claim 43.
- 46. (original) A host cell according to claim 45 wherein said vector exists within the host cell as a plasmid.

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- 47. (original) A host cell according to claim 45 wherein said vector is integrated into the genome of the host cell.
- 48. (currently amended) A method as claimed in <u>claim 20 any one of claims 20 to 32</u>-wherein the animal is a ruminant.
- 49. (original) A method as claimed in claim 47 wherein the animal is a sheep.
- 50. (currently amended) A method as claimed in <u>claim 33 any one of claims 33 to</u> 40 wherein the animal is a ruminant.
- 51. (original) A method as claimed in claim 50 wherein the ruminant is a sheep.